Detecting Cross-incompatibility of Three North American Apricot Cultivars and Establishing the First Incompatibility Group in Apricot

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Abstract. Laboratory and orchard tests have shown that the apricot (*Prunus armeniaca* L.) cultivars ‘Hargrand’, ‘Goldrich’, and ‘Lambertín-1’ are cross-incompatible. All three cultivars are from North American breeding programs and have ‘Perfection’ as a common ancestor. In orchard tests, compatible pollinations resulted in 19% to 74% fruit set, while incompatible pollinations resulted in <2% fruit set. Microscopic examination showed that, in incompatible pollinations, pollen tube growth was arrested in the style, most frequently in its third quarter, and that the ovary was never reached. It is proposed that self-incompatibility in apricot is of the gametophytic type, controlled by one S-locus with multiple alleles, and that these three cultivars are S₁S₂.

European apricot (*Prunus armeniaca*) cultivars have traditionally been considered self-compatible (Mehlenbacher et al., 1991). However, self-incompatible cultivars have been described by several researchers. Schultz (1948) pointed out that ‘Riland’ and ‘Perfection’, two cultivars traditionally grown in the United States, are self-incompatible. Later, many papers confirmed that, although apricot populations in Europe and North America were mainly self-compatible, self-incompatibility was more frequent than had been thought. Lamb and Stiles (1983) found five self-incompatible cultivars in a study of 19 cultivars suitable for cultivation in New York, and Nyújtó et al. (1985) found one case of self-incompatibility among 23 tested cultivars. Most recently, several self-incompatible cultivars were found among those traditionally grown in Spain (Burgos et al., 1993; Egea et al., 1991).

Self-incompatibility in *Prunus* appears to be controlled by a monogenic system with a multiallelic series (De Nettancourt, 1977). Data from almond (Dicenta and García, 1993; Socias i Company, 1991) and sweet cherry (Crane and Brown, 1937) are consistent with this hypothesis. Also, preliminary results indicate the same system operates in apricot (Burgos, 1995). The locus controls self-incompatibility and intraspecific cross-incompatibility (Kester et al., 1994; Socias i Company, 1991).

The increasingly narrow genetic base of breeding programs, a consequence of using a small number of cultivars as parents, has caused the frequent appearance of cross-incompatibility between cultivars of some species. Early work by Tufts and Philp (1922) established two incompatibility groups in almond. Much later, the work of Kester and collaborators made it possible to establish new incompatibility groups and assign additional cultivars to the groups described earlier (Kester, 1963; Kester and Asay, 1975; Kester et al., 1994). This was mainly due to the use of ‘Nonpareil’ and ‘Texas’ (‘Mission’) as common ancestors of many of these cultivars.

In apricot, only one case of cross-incompatibility has been reported between Spanish cultivars: ‘Moniquí Fino’ and ‘Moniquí Borde’ (Egea et al., 1991). The names of these cultivars indicate a

probable kinship. Burgos et al. (1993) did not find cross-incompatibility after crossing other self-incompatible cultivars from the same Spanish population. Many Spanish apricot cultivars seem to have originated from crosses between north African cultivars (mainly self-incompatible) and European cultivars (mainly self-compatible) (Egea et al., 1988). Hence, a great degree of heterozygosity is to be expected and self-incompatibility might be expected.

In the present work, cross-compatibility between some North American cultivars was studied by examining pollen tube growth using fluorescence microscopy and fruit set from controlled pollinations in the orchard.

Materials and Methods

Plant material. Studies were conducted in the apricot collection at the Departamento de Mejora y Patología Vegetal, CEBAS–CSIC, Murcia, Spain. Five-year-old trees of ‘Lambertín-1’, ‘Hargrand’, ‘Harcot’, and ‘Goldrich’ were used in this study. These cultivars were obtained from breeding programs in the United States and Canada. Five-year-old trees of the self-incompatible ‘Moniquí’ (Burgos et al., 1993) and the self-compatible ‘Canino’ and ‘Bebeco’ (Audergon et al., 1988) were also used to check experimental methodology.

The parentage of the cultivars used in this study is shown in Fig. 1. ‘Goldrich’ is a seedling from ‘Sun Glo’ × ‘Perfection’ (Toyama, 1971). ‘Sun Glo’ was developed by O.H. Heider as a seedling of unknown parentage, which originated in Washington in 1942 (Brooks and Olmo, 1972a). ‘Perfection’ was introduced in 1937 in Waterville, Wash. (Brooks and Olmo, 1972a), and has been used widely in American breeding programs despite its self-incompatibility. ‘Geneva’ and ‘Naramata’ are seedlings of unknown parentage. ‘Geneva’ originated in Italy and was planted, evaluated, and selected in New York by R. Wellington in 1934 (Brooks and Olmo, 1972a). ‘Lambertín-1’ was initially selected by the U.S. Dept. of Agriculture–Agricultural Research Service at Fresno, Calif., and evaluated under the designation P32-18.

Pollen collection and controlled pollinations. Flowers in the balloon stage were collected from all cultivars to be used as male parents. Anthers were removed from the flowers and placed in a petri dish. The anthers were desiccated in a petri dish in a calcium chloride desiccator for 24 h. then the pollen was stored in small bottles and kept at 4 °C.

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Fig. 1. Parentage of the apricot cultivars used in this study (Layne, Ledbetter, and Howell, personal communication). Unless indicated otherwise, the female parent is at the top and the male parent is at the bottom. OP = open-pollination.

For controlled crosses in the orchard, several branches with a total number of about 200 flowers in the balloon stage were chosen on each parent tree. Open flowers and immature buds were removed, and flowers at the balloon stage were emasculated to prevent self- and cross-pollination. Controlled pollinations were carried out using a small brush and the corresponding pollen from each male parent. After 8 weeks, fruit were counted and fruit set percentages were determined.

For laboratory testing, branches of the cultivars to be pollinated with about 30 flowers each in the balloon stage were cut and placed in plastic bags containing water. The bags were transported to the

laboratory in an insulated ice chest. Once in the laboratory, the basal ends were placed in beakers containing a 5% sucrose solution in a chamber where the temperature was maintained at 20 °C. The flowers were emasculated and 24 h later they were self- or cross-pollinated. Also, branches with a similar number of flowers in balloon stage were marked on the tree and the flowers were emasculated. After 24 h, the flowers were pollinated, for comparison with the laboratory controlled pollinations. Seventy-two hours after controlled pollination of the flowers transported to the laboratory and 192 h after controlled pollination of the flowers on the tree, 15 pistils per combination were harvested and immersed in FAA (90% alcohol at 70%, 5% formaldehyde at 40%, 5% glacial acetic acid) in small glass bottles. The pistils were stored at 4 to 5 °C in this solution. According to Williams (1970), 192 h is a sufficient time for the pollen tubes to reach the ovary in compatible

Table 1. Apricot pollen tube growth 72 h after self- and cross-pollinations under laboratory controlled conditions.^z

Pollinated cultivar	Pollinizing cultivar	Ovaries reached by pollen tubes (%)	Avg no. of pollen tubes in the ovary	Percent of style length penetrated by the longest pollen tube
Lambertin-1	Lambertin-1	0 ± 0.0	0 ± 0.0	76.1 ± 8.0
	Goldrich	0 ± 0.0	0 ± 0.0	86.3 ± 6.1
	Hargrand	0 ± 0.0	0 ± 0.0	69.7 ± 16.7
	Harcot	100 ± 0.0	6.2 ± 3.2	100 ± 0.0
Goldrich	Goldrich	0 ± 0.0	0 ± 0.0	72.5 ± 11.9
	Lambertin-1	0 ± 0.0	0 ± 0.0	54.3 ± 12.3
	Hargrand	0 ± 0.0	0 ± 0.0	82.9 ± 9.0
Hargrand	Harcot	100 ± 0.0	11.4 ± 1.5	100 ± 0.0
	Hargrand	0 ± 0.0	0 ± 0.0	69.2 ± 9.8
	Lambertin-1	0 ± 0.0	0 ± 0.0	---
	Goldrich	0 ± 0.0	0 ± 0.0	74.8 ± 9.9
Harcot	Harcot	100 ± 0.0	3.9 ± 2.0	100 ± 0.0
	Harcot	0 ± 0.0	0 ± 0.0	53.9 ± 12.7
	Lambertin-1	100 ± 0.0	3.7 ± 2.0	100 ± 0.0
	Goldrich	100 ± 0.0	7 ± 2.9	100 ± 0.0
	Hargrand	100 ± 0.0	4.2 ± 1.9	100 ± 0.0

^zValues are means from 10 samples ± sd.

Table 2. Fruit set percentages obtained from controlled self- and cross-pollinations of apricot cultivars.

Pollinated cultivar	Pollinizing cultivar	Fruit set (%)
Lambertín-1	Lambertín-1	1.7
	Harcot	73.7
	Hargrand	1.9
	Goldrich	0
Hargrand	Hargrand	0
	Harcot	19
	Lambertín-1	0
	Goldrich	0
Harcot	Harcot	0
	Goldrich	62.5
	Lambertín-1	65.9
	Hargrand	36.7
Goldrich	Goldrich	0

combinations at the daily average temperatures of about 15 °C occurring during our orchard pollinations. To remove the FAA, the pistils were rinsed in distilled water for three times 1 h each, and then placed in an autoclave for 30 min at 1 atmosphere in a 5% sodium sulfite solution to soften the tissues and facilitate their staining with 0.1% aniline blue in 0.1 N potassium phosphate. The pistils were stained for 24 h. The epidermis was then removed, and the pistils squashed for observation (Linskens and Esser, 1957).

An Olympus BH2 microscope (Olympus, Tokyo) was used with a BH2-RFL-T2 ultraviolet light source, using an Osram HBO 100 W/2 high-pressure mercury lamp (Osram GmbH, Berlin-Munich).

Results

Ten of the sixteen pollinations performed in the laboratory were incompatible (Table 1). In incompatible crosses, pollen tube growth was arrested about three-fourths of the way down the style. The percentages of fruit set following self-pollination confirm that 'Goldrich', 'Hargrand', 'Harcot', and 'Lambertín-1' are self-incompatible (Table 2) and that the first three are cross-incompatible in all possible combinations. 'Harcot' was compatible with the other three cultivars. No pollen tube reached the ovary in the six different combinations between 'Lambertín-1', 'Hargrand', and 'Goldrich' (Table 1). Orchard cross-pollination confirmed the laboratory results and no fruit set was obtained in those combinations tested except for very low fruit set (1.9%) in the combination 'Lambertín-1' x 'Hargrand' (Table 2). Results of the incompatible combinations (self- and cross-) strongly contrast, in laboratory and field pollinations, with those from the compatible ones, where 100% of the ovaries had a high average number of pollen tubes (Table 1) and large percentages of fruit set were obtained (Table 2).

Some differences were found in the length of the style that pollen tubes were able to reach in the incompatible combinations. The percentages of style reached by the longest pollen tube ranged from 48.8% in the cross 'Goldrich' x 'Lambertín-1' to 86.3% in the reciprocal cross. These differences are probably genotype-combination dependent. Table 3 shows self-compatible and self-incompatible cultivars that were self-pollinated in the orchard to assess results from laboratory controlled-pollinations. Very clear differences in the growth of pollen tubes were seen. In self-compatible cultivars, pollen tubes grew the entire length of the style and entered the ovary, while in self-incompatible cultivars tube growth stopped in different parts of the style, depending on the cultivar.

Pollen tube growth in flowers pollinated in the field and collected and fixed 192 h later was the same as on flowers pollinated in the laboratory (Table 3).

Discussion

The three methods used to determine compatibility correlate well and may be used interchangeably (Burgos et al., 1993), although laboratory pollinations provide more controlled conditions and help avoid uncontrolled cross-pollinations and unfavorable weather conditions.

The self-incompatibility of 'Goldrich' was previously described by Lamb and Stiles (1983). No information on 'Harcot's' self-incompatibility was given when the cultivar was released (Layne, 1978) or by Lamb and Stiles (1983), who nevertheless included 'Harcot' in a study of apricot cultivars suitable for cultivation in New York, where they described this trait for other apricot cultivars. In contrast with our results, 'Hargrand' was described as self-compatible when released (Layne, 1981) as was 'Lambertín-1' (Audergon et al., 1988).

The first reported case of cross-incompatibility in apricot was described between 'Moniquí Fino' and 'Moniquí Borde' (Egea et al., 1991). However, their names and morphological similarity indicate that they may be slightly different clones of the same cultivar.

The self- and cross-incompatibility results cannot be explained by a lack of pollen germination, since pollen germination took place in all crosses and pollen tube growth was arrested in the style. Also, all cultivars when crossed with 'Harcot' as female parent showed good pollen germination on the stigma (data not shown), and the number of pollen tubes reaching the ovary was high, as was the percentage of fruit set.

The low percentage of fruit set obtained after self-pollination of 'Lambertín-1' (1.7%) and the combination 'Lambertín-1' x 'Hargrand' (1.9%) may have been caused by bees visiting the flowers, despite the flowers' being emasculated (Williams et al., 1984). It could also be a case of pseudocompatibility, which has been reported as a nongenetic condition that is environmentally influenced by external (i.e., temperature) or internal (i.e., flower age) conditions (Van Gastel, 1976). Examples of pseudo-compatibility include young flower buds that have not yet developed the incompatibility barriers or older flowers in which these are no longer effective (Van Gastel, 1976; Williams and Maier, 1977). Similar results have been obtained artificially by keeping the flowers at specific temperatures during pollen tube growth (Williams and Maier, 1977) or by heating the pistil at specific periods (Hiratsuka et al., 1989).

A model for the inheritance of incompatibility in *Prunus* has

Table 3. Percentage of pistil reached by the longest pollen tube 192 h after self-pollination of self-compatible and self-incompatible apricot cultivars in field conditions.²

Self-pollinated cultivar	Percentage of style penetrated by the longest pollen tube
Self-incompatible cultivar	
Lambertín-1	69.2 ± 10.3
Harcot	57.7 ± 17.5
Moniquí	81.8 ± 7.6
Self-compatible cultivar	
Bebeco	100 ± 0.0
Canino	100 ± 0.0

²Values are means from 10 samples ± SD.

been proposed as a homomorphic, monofactorial, and gametophytic system (De Nettancourt, 1977). Preliminary results in apricot (Burgos, 1995) seem to confirm this hypothesis, where a locus with a multiallelic series would control the trait. Hence, cross-incompatible cultivars would have the same genotype for the trait. We propose designating those two alleles as S_1 and S_2 .

The three cross-incompatible cultivars have 'Perfection' among their ancestors. This is an old cultivar used extensively in North American breeding programs because of its large size, attractive orange color, and firm flesh. 'Perfection' is a parent or grandparent of some recently introduced cultivars such as 'Rival' (Brooks and Olmo, 1971), 'Tracy' (Brooks and Olmo, 1972b), 'Skaha', 'Sundrop', and 'Westley' (Brooks and Olmo, 1975) or 'Castlebrite' (Brooks and Olmo, 1978). It would be interesting to identify the alleles in 'Perfection', but unfortunately this cultivar was not in our apricot collection. 'Goldrich' (S_1S_2 , as proposed) and 'Perfection' (S_1S_3) have an allele in common since 'Goldrich' was obtained from the cross 'Sun Glo' x 'Perfection'. If 'Goldrich' and 'Perfection' are cross-compatible, seedlings from this cross would be of two different genotypes (i.e., two incompatibility groups) since S_1 alleles would be arrested. One of these groups would be the same as the male parent (S_1S_2 or S_1S_3 if 'Goldrich' or 'Perfection' respectively, are the male parent), while the other one would be new (S_2S_3). These cultivars may also belong to the same incompatibility group and be cross-incompatible if 'Perfection' and 'Sun Glo' share one allele. Crosses of 'Goldrich' with unrelated cultivars would give genotypes representing four incompatibility groups. It is only by making controlled crosses and identifying incompatibility groups in these progenies that alleles can be assigned to groups.

The narrow genetic base resulting from using a limited number of parents has resulted in a small number of incompatibility groups in almond or cherry (Kester et al., 1994). Incompatibility is difficult to detect and is also undesirable, since incompatible clones require pollenizers and render the yield dependent on abundant pollen transfer among the trees (Frankel and Galun, 1977). The appearance of cross-incompatibility in this group of cultivars indicates that greater attention should be given to this character in breeding programs. The laboratory pollination procedure described here would allow quick and easy identification of self-incompatible genotypes.

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